HIGH ACCURACY 5-PART DIFFERENTIAL WITH DIGITAL HOLOGRAPHIC MICROSCOPY AND UNTOUCHED LEUKOCYTES FROM PERIPHERAL BLOOD

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This is a 371 of PCT/EP2017/051656, filed Jan. 26, 2017, which claims priority to European Patent Application No. EP 16182979.1, filed Aug. 5, 2016, and European Patent Application No. EP 16160664.5, filed Mar. 16, 2016, each of which is hereby incorporated by reference herein in its entirety for all purposes.

FIELD

[0002] The present invention relates to an improved method for marker-free detection of a cell type of at least one cell in a medium using microfluidics and digital holographic microscopy, as well as a device, particular for carrying out the method.

BACKGROUND

[0003] One essential part of blood cell in-vitro diagnostics is the differentiation and counting of white blood cells (WBC). The WBC cell types are usually stated as lymphocytes, monocytes and granulocytes, which are referred to as 3-part differential of the WBC in a hematological test result. Granulocytes can be further divided into 3 types of granulocytes—neutrophils, eosinophils, and basophils. Hence, a 5-part differential refers to the 5 main white blood cell types of the peripheral blood.

[0004] With label-free methods, such as Mie scatter analysis, usually a 3-part differential is feasible, which does not allow a discrimination of the types of granulocytes. Mie scatter of individual target cells at a low and high angle is usually used to discriminate WBC for a 3-part differential. To minimize scattering of red blood cells hemolysis of the erythrocytes is performed. For a 5-part differential result one requires additional labeling of the cell nucleus and granulae to discriminate eosinophils and basophils from neutrophils. Additional labeling allows a further discrimination of the granulocytes for a 5-part differential based on scatter and absorption measurements. This method can be automated and used in hematology analyzers working as a flow cytometers.

[0005] In general, hematology analysis is performed with automated hematology analyzers, and—in case of ambiguous results (flagged results)—microscopy of Giemsa stained blood samples on a microscope slide is performed for a manual differential diagnosis. The disadvantage of automated hematology analyzers is the requirement for defined gates for data analysis, which leads to flagged samples in cases where pathologies lead to a shift of the scatter information beyond the limits of the gates. In addition, with a hematology analyzer the user has no image information which can allow X-differential information of the WBC, such as platelet-leucocyte aggregates or pathological morphologies.

[0006] Similar limitations exist for microscopy analysis of fixed and, e.g., panchromatically, stained cells on microscopy slides, which is usually limited to the analysis of 100-200 WBC maximum. However, in pathological cases WBC cell concentrations of interest can span more than five orders of magnitudes (0.1-10.000 cells/µl), which require a large blood volume for sufficient statistics on the different WBC populations. Further, Giemsa stained samples require fixation, drying and panchromatic staining of whole blood samples on a microscope slide, i.e., additional analysis steps.

[0007] Overall, a large dynamic concentration range cannot be covered by blood smear analysis having low statistical power. In addition, hematology analyzers based on impedance or Mie scatter analysis cannot provide morphological information.

[0008] U.S. Pat. No. 5,017,497 of deGrooth, Terstappen et al used as the basis for hematology counters a plot of depolarized vs. polarized side scatter discriminates unfixed, unstained eosinophil granulocytes from neutrophils, which is also described in SHAPIRO, HOWERD M.: "Practical Flow Cytometry", 4th Ed., pp. 278-9 and FIG. **7-2**; 3rd Ed., pp. 236-7 and FIG. **7-2**. However, in general staining is the reference method and is applied for 5-part Diff.

[0009] Further, digital holographic microscopy (DHM) has been described with regard to leukocyte discrimination, e.g., in DE 10 2014 200 911 A—incorporated herein with reference regarding leukocyte discrimination using digital holographic microscopy; VERCRUYSEE, DRIES et al.: "Three-part differential of unlabeled leukocytes with a compact lens-free imaging flow cytometer" in: Lab Chip., 2015, Vol. 15, pp. 1123—reporting a 3 part differential with DHM using fixed cells; and US 2014/0220622 A1—reporting on DHM for leukocyte differentiation without specifying how a 5 part differential can be achieved.

[0010] However, there is a need for an improved and reliable, label-free, preferably quantitative method of discriminating white blood cells in high numbers, particularly in higher throughput.

SUMMARY

[0011] The present inventors optimized a microscopic approach using microfluidics to solve the above problems and obtain a system for online monitoring and differentiating blood cells in samples of peripheral blood.

[0012] In a first aspect, the present invention relates to a method for marker-free detection of a cell type of at least one cell in a medium, comprising flowing a medium comprising at least one cell into a microfluidic device, obtaining an image of the at least one cell in the microfluidic device by a digital holographic microscopic device, wherein the image is obtained with a depth of field of less than 6 µm, and determining the cell type of the at least one cell.

[0013] The invention further relates to a device for marker-free detection of a cell type of at least one cell in a medium, comprising

[0014] a digital holographic microscopic device with a depth of field of less than 6 µm;

[0015] a microfluidic device; and

[0016] a detection system configured to determine the cell type of the at least one cell.

[0017] Further aspects and embodiments of the invention are disclosed in the dependent claims and can be taken from the following description, figures and examples, without being limited thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The enclosed drawings should illustrate embodiments of the present invention and convey a further under-